

Applicant : LEUNG, Shawn Shui-on  
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**REMARKS**

Claims 1-24 were pending in this Application. Applicant canceled claims 16-19 without prejudice to the Applicant's rights to pursue the subject matters in a future application. Claims 14-15 and 20-24 were withdrawn. By this Amendment, Applicant canceled claims 1-13 without prejudice to the Applicant's rights to pursue the subject matters in a future application.

Applicant submitted new claims 25-39 for consideration by the Examiner. Applicant maintains that new claims 25-39 are well supported *inter alia* by the specification as originally filed and there is no issue of new matters. Accordingly, Applicant respectfully requests the entry of this Amendment. Upon entry of this Amendment, Claims 25-39 will be pending and under examination.

**Rejections Withdrawn**

Applicant acknowledges that the rejection of claims 1-13 under 35 U.S.C. § 112, second paragraph, for parts (b), (c), (d), (g)-(i) in the last [January 7, 2003] office action has been withdrawn by the Examiner.

Applicant further acknowledges that the rejection of claims 16-19 under 35 U.S.C. § 112, first paragraph, [in the January 7, 2003 Office Action] has been withdrawn by the Examiner.

**Response to Arguments**

The Examiner rejected claims 1-13 under 35 U.S.C. § 102(b) as being anticipated by Queen et al. (U.S. Patent No. 5,693,762, issued 12/97). The Examiner stated that the rejected is maintained because "the art of Queen et al. reads on the claims."

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In response but without conceding the correctness of the Examiner's position and to expedite the prosecution of this Application, Applicant has canceled claims 1-13 without prejudice. New claims 25-39 recite "not all the replaced FR1, FR2, FR3 and FR4 of the re-engineered immunoglobulin heavy chain are from the same framework of a single immunoglobulin heavy chain; whereas not all the replaced FR1, FR2, FR3, and FR4 of the re-engineered immunoglobulin light chain are from the same framework of a single immunoglobulin light chain", thereby rendering this ground of rejection moot. (Also see section under the heading "Rejection under 35 U.S.C. § 102" below).

**Rejection under 35 U.S.C. § 112, Second Paragraph**

The Examiner rejected claims 1-13 under 35 U.S.C. § 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention."

a. The Examiner stated that "claims 1-13 are indefinite for reciting 'heavy and/or light chain' in claim 1, line 2 and 'heavy and light chain' in claim 1, line 6-7 because it is unclear if the claims use both heavy and light chains or one or the other."

In response but without conceding the correctness of the Examiner's position and to expedite the prosecution of this Application, Applicant has canceled claims 1-13 without prejudice. New claims 25-39 recite "heavy or light or heavy and light chain", thereby rendering this ground of rejection moot.

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b. The Examiner further stated that there is insufficient antecedent basis for "said re-engineering immunoglobulin chain(s)" in claim 1.

In response but without conceding the correctness of the Examiner's position and to expedite the prosecution of this Application, Applicant has canceled claims 1-13 without prejudice. New claims 25-39 do not recite "said re-engineering immunoglobulin chain(s)", thereby rendering this ground of rejection moot.

c. The Examiner further stated that "claim 1 and those depended on claim 1 is indefinite for reciting 'different immunoglobulin chains' because it is unclear if the frameworks are from the light chain and substituted for the heavy chain or visa versa or heavy chain are substituted for heavy and light for light chains or that the frameworks are from the light chain of one source and the frameworks from the heavy chain are from a different source."

In response but without conceding the correctness of the Examiner's position and to expedite the prosecution of this Application, Applicant has canceled claims 1-13 without prejudice. New claims 25-39 recite "a re-engineered... immunoglobulin containing heavy or light or heavy and light chain variable region sequences... in which at least one of the compartmentalized framework sequences... are replaced, or patched by the corresponding framework sequences from the heavy or light or heavy and light chain immunoglobulin variable region..., respectively...", thereby rendering this ground of rejection moot.

d. The Examiner further stated that "claims 6-9 are indefinite for reciting 'which is the particular FR derived

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from a different source used for patching or that replaces the original FR of, the parent immunoglobulin' because it is unclear what the FR is from.

In response but without conceding the correctness of the Examiner's position and to expedite the prosecution of this Application, Applicant has canceled claims 1-13 without prejudice.

New claims 25-39 recite "wherein the patching FR is the FR derived from a different source used for patching or that replaces the original FR". The FR referred to is the one used to patch the corresponding FR of the parent antibody of the corresponding chain, thereby rendering this ground of rejection moot.

e. The Examiner further stated that "claims 1-13 are indefinite for reciting 'within ten-fold or within 3-fold' in claim 1..."

In response but without conceding the correctness of the Examiner's position and to expedite the prosecution of this Application, Applicant has canceled claims 1-13 without prejudice. New claims 25-39 recite "within 10-fold or within 3-fold". The element "within 10-fold" can be found on page 2, line 10 of the Specification as originally filed thereby rendering this ground of rejection moot.

**Rejection under 35 U.S.C. § 112, First Paragraph**

The Examiner rejected claims 1-13 under 35 USC 112, first paragraph, "as failing to comply with the written description requirement."

The Examiner further stated:

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The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.. Claim 1 has been amended to recite "within ten-fold". The response filed 12/9/03 did not state where support for the limitation can be found. The examiner found support for within 3-fold on page 12 but did not apparently find support for the within 10-fold limitation. Applicant is required to provide specific support for the limitation or remove it from the claim.

In response, Applicant respectfully traverses the Examiner's above ground of rejection. Applicant maintains that support for "within ten-fold" can be found on page 2, line 10 of the Specification as originally filed.

The Examiner set forth the following factors for determining whether undue experimentation is required, citing a case: "nature of the invention, the state of the art, the relative skill of those in the art, the amount of direction or guidance disclosed in the specification, the presence or absence of working examples, the predictability or unpatentability of the art, the breadth of the claims, the quantity of experimentation which would be required in order to practice the invention as claimed."

The Examiner further stated:

While being enabling for a re-engineered antibody comprising a heavy chain and a light chain wherein at least one of FR1, 2, 3, 4 in the heavy chain or the light chain or both are replaced with the FR of the heavy or the light chain or both from the corresponding FR from a different species, wherein the antibody binds an antigen within three-fold of the parent antibody and wherein that not all of the replaced FR1, 2, 3, and 4 are from the same framework from a single immunoglobulin chain, does

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not reasonably provide enablement for a reengineered antibody that has FR replaced in a heavy chain with those from another species in a light chain or vice versa wherein the antibody binds antigen.

In response but without conceding the correctness of the Examiner's position and to expedite the prosecution of this Application, Applicant has canceled claims 1-13 without prejudice. New claims 25-39 recite "not all the replaced FR1, FR2, FR3 and FR4 of the re-engineered immunoglobulin heavy chain are from the same framework of a single immunoglobulin heavy chain; whereas not all the replaced FR1, FR2, FR3 and FR4 of the re-engineered immunoglobulin light chain are from the same framework of a single immunoglobulin light chain". The claims do not recite using FRs from a heavy chain to swap with FRs of a light chain, thereby rendering this ground of rejection moot.

#### **Rejection under 35 U.S.C. § 102**

The Examiner rejected claims 1-13 under 35 U.S.C. § 1-2(b) as being anticipated by Ohtomo et al. (Molecular Immunology 32:407-416, 1995).

The Examiner stated:

Ohtomo et al teach a reengineered antibody from a parent wherein FR4 is from the ND human antibody and the FR1-3 are from the EU human antibody (see abstract and Figure 2) wherein residues close to a CDR or indicated to influence antigen binding (from a molecular model, see page 408) are reintroduced into the FR (see abstract and page 412), wherein the reengineered antibody binds antigen essentially with the same affinity as the parent (see Figure 3), wherein the antibody is pure and comprises BSA (see page 410).

Ohtomo et al is silent as to the affinity of the antibody or whether the residues are within 4

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angstroms of a CDR, however, it is the examiners position that the antibody of Ohtomo et al has the same affinity and residues that are reintroduced are within 4 angstroms of a CDR as that claimed. One of ordinary skill in the art would reasonably conclude that Ohtomo et al antibody also possesses the same structural and functional properties as those of the antibodies claimed and, therefore, it appears that Ohtomo's antibodies are identical to the claimed antibody. Since the Patent and Trademark Office does not have the facilities for examining and comparing the claimed antibody with the antibody of Ohtomo, the burden of proof is upon the Applicants to show a distinction between the structural and functional characteristics of the claimed antibody and the antibody of the prior art.

In response, Applicant respectfully traverses the Examiner's above ground of rejection.

Applicant's claim 1 recites:

A re-engineered, or framework (FR)-patched immunoglobulin containing a heavy or light or heavy and light chain variable region sequences from a parent antibody, in which at least one of the compartmentalized framework sequences, defined as FR1, FR2, FR3 and FR4 are replaced, or patched by the corresponding framework sequences from the heavy or light or heavy and light chain immunoglobulin variable region of a different species, respectively, wherein said re-engineered immunoglobulin comprises framework sequences derived from at least two different sources of different immunoglobulin chains, wherein said different immunoglobulin chains can be sourced from different immunoglobulins of the same species or from different immunoglobulins of different species, and such re-engineered immunoglobulin binds specifically to an antigen with affinity within 3-fold of, that of the parent immunoglobulin with the proviso that not all the replaced FR1, FR2, FR3 and FR4 of the re-engineered immunoglobulin heavy chain are from the same framework of a single immunoglobulin heavy chain; whereas not all the replaced FR1, FR2, FR3 and FR4 of the re-engineered immunoglobulin light chain are from the same framework of a single immunoglobulin light chain.

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See Table I below for a comparison of the differences between the present invention and Ohtomo et al. or Queen et al.

**Table I. Comparison of the Differences between the Present Invention and Prior Art**

<b>Framework Patching</b> Leung Application	<b>Humanization</b> Ohtomo et al. / Queen et al.
FR segments which are chosen separately and independently, e.g. FR1, FR2, FR3 and FR4 are replaced, or patched by the corresponding framework sequences from the heavy or light or heavy and light chain immunoglobulin variable region of a different species, respectively.	Consider the whole framework sequence in its entirety without altering the FR combination within a single VH gene or a single VK gene.
Genetic structure of the immunoglobulin variable regions looks un-natural, or potentially foreign to the immune system when viewed from a 3D perspective.	Mimic the physical appearance of naturally occurring human antibodies.
Minimize the numbers of potential B and T epitopes through compartmentalization or segmentation of each individual FR segments by creating an UN-NATURAL immunoglobulin.	Create new B- and T-epitopes by inclusion of re-introduced murine residues.
Functionally, the resultant antibody is viewed by the immune system (through MHC I and MHC II presentation) as self or human.	Re-introduced murine residues may create new B- and T-epitopes, and the resultant antibody can be viewed by the immune system (through MHC I and MHC II presentation) as functionally foreign and non-human.
Select segments of frameworks from the human database that would have IDENTICAL or CONSERVATIVELY SIMILAR residues at positions that are adjacent to CDR or being rare in the parent antibody.	Re-introduction of the murine residues that are adjacent to CDR or being rare in the parent antibody.
Re-introduce murine residues only if framework patching is insufficient in maintaining the original immunoreactivities to a preset range.	

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Please see **Exhibit A** for Diagrams Illustrating the Differences between Framework-Patching and Humanization.

Applicant maintains that even if Ohtomo et al. reported the use of the framework sequence of a single VH gene (i.e. FR1, FR2 and FR3) from one antibody, and the FR4 from a different antibody in the construction of a heavy chain variable region, Ohtomo et al. does not anticipate the present invention. The disclosure in Ohtomo et al. or Queen et al. or in combination cannot enable one of ordinary skill in the art to come up with a similar invention.

Applicant further maintains that antibody genes are assembled from a series of discontinuous germ-line gene segments that are juxtaposed during B-lymphocyte development. For human antibody heavy chains, a series of several hundred variable (VH) region gene segments (less than 100 are functional), 25 to 30 functional DH segments, and six functional JH segments contribute to the generation of a diverse repertoire of antigen binding sites. It is understood that the FR1, FR2 and FR3 segments all reside within a single VH gene, whereas the DH gene constitutes part of the CDR3, and the JH gene constitutes part of the CDR3 and all of the FR4 (the same rationale applies to the variable region portion with the VL gene constituting the FR1, FR2 and FR3 segments, and the JL gene constituting the FR4 segment). Unlike the FR4 which is contained within the JH gene, the FR1, FR2, and FR3 of a single VH gene are structurally and functionally inseparable in a natural environment. The use of the FR1, FR2 and FR3 from EU (VH gene) and FR4 from ND (JH gene) for CDR-grafting is actually mimicking the natural gene shuffling mechanism in forming a naturally antibody. The FR1, FR2 and FR3 are still left intact as from one single VH gene segment. Given the

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limited number of functional JH genes (six in total), it is safe to assume that the assortment of the particular VH genes with any one of the six JH segments will occur in natural human antibodies, and thereby the combination of the EU VH gene with the ND JH gene should have occurred in natural situation (in choosing the EU/ND combination, in page 412, Ohtomo et al. was careful to point out that both EU and ND belong to the same subgroup 1, presumably in an attempt to justify the combination as natural) even though natural sequence with EU FR1, FR2 and FR3, and ND FR4 could not be identified in current database such as the Kabat database.

Nevertheless, the idea of Ohtomo et al. has not deviated from the original rationale for humanization as taught by Queen et al., by adopting the framework from a natural human immunoglobulin which is expected to be present and tolerated in human (given that the current database has approximately 1200 human VH sequences, which is very limited).

Applicant further maintains that the publication by Ohtomo et al. does not anticipate the invention of the current application. The basic idea was still humanization similar to that of Queen et al. by taking into consideration of the FRs (or the antibody structure) in its entirety without altering the FR combination within a single VH gene; and their ideas of reducing immunogenicity were to make the V region look as similar as possible from a 3D perspective to a natural human antibody. One with ordinary skill in the art, after reading Ohtomo et al. or Queen et al. or in combination, would not be able to venture into contemplating assorting FR1, FR2 and FR3 from different antibody sources into the construction of a V region because this is against conventional understanding on the genetic structure and function of the immunoglobulin variable regions, and they will look un-natural, or potentially foreign to the immune system when viewed from a 3D

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perspective. Applicant maintains that "framework patching" cannot be invented without spending enormous time on literature reviews, experimentations, and theorizing, even after Applicant had published the construction of a hybrid VH (Leung et al. 1995. Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific, leukemia/lymphoma antibody, LL2. Mol. Immunol. 32:1413-1427) similar to that of Ohtomo et al.

Applicant further maintains that the biggest difference between humanization as taught by Queen et al. and framework patching is that humanization deals with the problem of immunogenicity by making the V region to look as similar as possible in "appearance" to a natural immunoglobulin, whereas framework-patching deals with the problem of immunogenicity by aiming to minimize the numbers of potential B and T epitopes through compartmentalization or segmentation of each individual FR segments by creating an UN-NATURAL immunoglobulin through swapping and assorting FR1, FR2, FR3 from different immunoglobulins with different VH sequences, especially when viewed from a gene structure level. The Ohtomo's antibodies are therefore **NOT** structurally identical to the claimed antibody.

Applicant further maintains that from a functional perspective, since a framework-patched antibody aims to eliminate or reduce immunogenicity by first mimicking the humanness of each segment of framework independently, even when these segments are structurally linked and considered functionally inseparable within a single VH gene, in order to avoid the need to introduce murine residues and functionally "create" potentially new B- and T-epitopes. The physical appearance of the resultant antibody may not look naturally humanized, but functionally, it is viewed by the immune system (i.e. after being internalized and the FR segments digested

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and presented as peptides in the context of MHCI and MHCII) as self or human. Whereas in the case of Ohtomo's antibodies, they mimic the physical appearance of naturally occurring human antibodies, but functionally, since they encourage the inclusion of re-introduced murine residues (compared to the present invention of finding the appropriate segment of framework so that the number of re-introduced murine residues can be reduced or eliminated), which would result in the possibility of creating new B- and T-epitopes, the resultant antibody can be viewed by the immune system as functionally foreign and non-human. Therefore, the claimed antibody is functionally different from that of Ohtomo et al.

Based on the above analysis and discussion, neither Ohtomo et al. nor Queen et al. teaches or discloses the Applicant's claimed invention. Accordingly, neither Ohtomo et al. nor Queen et al. anticipate the claimed invention. Therefore, Applicants respectfully request the reconsideration and withdrawal of this ground of rejection.

**Rejection under 35 U.S.C. § 103**

The Examiner rejected claims 1-13 under 35 U.S.C. § 103(a) "as being unpatentable over Ohtomo et al. (Molecular Immunology 32:407-416, 1995) and further in view of Queen et al (US Patent 5,693,762, issued 12/97, IDS #11/2)."

The Examiner set forth the following test for determining obviousness under 35 U.S.C. § 103(a), citing a case:

1. Determining the scope and contents of prior art
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence of nonobviousness

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In response, Applicant respectfully traverses the Examiner's above ground of rejection. Applicant maintains that, first, the scope and content of the scope and contents of the prior art were not indicative and suggestive of the current invention, including those published by Queen et al. and Ohtomo et al., as clearly explained above. (See section under the heading "Rejection under 35 U.S.C. § 102" above). Second, there are major differences both structurally and functionally between the method of framework-patching and the humanization methods of Ohtomo/Queen et al., as explained above. (See section under the heading "Rejection under 35 U.S.C. § 102" above). Moreover, Queen et al teach methods in choosing FR residues that are considered important in maintaining the binding affinity of the antibody, and then RE-INTRODUCE these residues back to the human framework, and of course this approach would have the potential of aggravating the immunogenicity problem by creating new B and T epitopes, and the problem was never addressed. In framework-patching, human sequences that have identical or conservatively similar residues at those positions are identified so that the re-introduction of murine residues into the selected segment of framework can be eliminated or minimized. Third, one with ordinary skilled in the art will not be able to conceive the present invention without a conceptual shift and extensive studies; that is exemplified by the Applicant who is skilled in the art and had independently done humanization using approach similar to that of Ohtomo et al. (at the same time or earlier, the Applicant constructed the humanized LL2 in 1993 and did not publish it until 1995) and came up with the idea of framework patching after Applicant has studied the intricate relationship between antigen presentation and immunogenicity over the course of his career.

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The Examiner further stated:

Ohtomo et al does not teach replacement of more than two FR from a different source wherein more than two FR would not be from the same immunoglobulin chain or that the reintroduced residues are at least 60% homology to the parent or residues are adjacent to CDR or replacing rare residues. This deficiency is made up for in the teaching of Queen et al.

In response, Applicant respectfully traverses Examiner's above ground of rejection. Applicant maintains that Ohtomo et al. does not teach replacement of more than two FRs from a different source wherein more than two FRs would not be from the same immunoglobulin chain and this deficiency is NOT made up for in the teaching of Queen et al.

The present invention teaches SELECTING segments of frameworks from the human database that would have IDENTICAL or CONSERVATIVELY SIMILAR residues at positions that are adjacent to CDR or being rare in the parent antibody (this has not be taught or implied or suggested by Ohtomo's publication), whereas Queen et al. teaches RE-INTRODUCTION of the murine residues that are adjacent to CDR or being rare in the parent antibody. Queen's teaching has not made up for the deficiency.

In the art of framework-patching, the idea is to eliminate the need for re-introducing murine residues back to the re-engineered antibody whereas Queen et al. teaches HOW TO SELECT THE MURINE RESIDUES TO BE REINTRODUCED BACK TO THE HUMANIZED SEQUENCE (although the invention does not preclude the reintroduction of murine residues back to the framework sequence when such needs are reduced to a minimum after framework patching).

The Examiner further stated:

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Queen et al teach humanization of antibodies wherein the FR are compared to human FR and the FR chosen are at least 70% homology to the parent and residues that are reintroduced are within 3 angstroms of a CDR or influence binding or interact with a CDR or is rare at that position and the affinity should be within two fold of the parent (see column 2-3).

In response, Applicant respectfully traverses Examiner's above ground of rejection. One major embodiment of the current application is that FR segments which are chosen separately and independently will have the sequence that are within 3 angstroms of a CDR or influence binding or interact with a CDR or is rare to be IDENTICAL or CONSERVATIVELY SIMILAR to that of the murine parent at the same position so that there will be NO NEED to reintroduce murine residues at such positions as in the case of Queen's humanization method. This embodiment does not read into that of Queen et al. That is the main difference between Queen's humanization and the method of framework patching, although the latter does not exclude the possibility of re-introducing murine residues at such positions, only if framework patching without changing the selected FR segment sequences is insufficient in maintaining the original immunoreactivities to a preset range. The possibility of re-introducing murine residues after framework patching previously described constitutes another embodiment of the present invention.

The Examiner further stated:

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced the claimed invention of replacing FR with human FR from any human antibody that has the most homology and re-introducing residues that influence binding in view of Ohtomo et al. and Queen et al.

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In response, Applicant respectfully traverses Examiner's above ground of rejection. Applicant maintains that, as explained earlier, the Queen et al. publication will not make it prima facie obvious to swap FR segments from different sources, especially at the FR1, FR2 and FR3 segments. From the teaching of Queen et al. and Ohtomo et al., as well as based on the training of a scientist skilled in the art of antibody engineering, the V region, and by implication the FR1, FR2 and FR3 contained within the VH gene or the VL gene, should be considered intact and inseparable. The homology Queen et al. refers to is restricted to the whole framework sequence in its entirety, instead of to be considered as independent segments. In choosing a whole framework sequence from a single source, Queen's approach will have to make modifications at specific locations of the framework (back-mutation) in order to maintain binding affinity. However, in a first embodiment of the present invention, the main purpose is to use a free assortment of FR segments to eliminate the need of such modifications, while maintaining the binding affinity. See Exhibit A. This first embodiment does not involve back-mutation and should have nothing related to any of Queen's teaching. Queen et al. teach methods to identify residues that have to be back-mutated, or reintroduced back to the humanized framework. A second embodiment is built on a framework-patched sequence and allows for the inclusion of back-mutated sequence (such back-mutation will be brought down to a bare minimum compared to the teachings of Queen et al) ONLY WHEN the framework-patched sequence is insufficient in maintaining affinity.

As explained earlier, Ohtomo's teaching of allowing the joining of FR4 from one human immunoglobulin with the FR1, FR2, FR3 of another human immunoglobulin does not make the intellectual transition to the invention of framework-patching

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prima facie obvious, in light of the gene structure of antibodies and the natural mechanism of gene reshuffling for the generation of diversity for antibodies.

The Examiner further stated:

One of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced the claimed invention of replacing FR with human FR from any human antibody that has the most homology and reintroducing residues that influence binding in view of Ohtomo et al and Queen et al because Ohtomo et al teach antibodies with FR from two human antibodies wherein the FR were chosen for highest homology with the parent FR.

In response, Applicant respectfully traverses Examiner's above ground of rejection. Applicant maintains that since the concept of framework patching is not prima facie obvious, as explained earlier, one of ordinary skill in the art would not be able to conceive the idea of selecting the human FR segments from the database that would have IDENTICAL or CONSERVATIVELY SIMILAR residues at critical positions separately and independently (Queen's teaching dictates the selection of a whole set of framework sequence for CDR-grafting, and in most cases, it is not possible to find one single framework with identical or conservatively similar residues at all or most critical positions, and thereby the need for back-mutation), especially between the FR1, FR2 and FR3 segments which are considered to be functionally and structurally inseparable. The homology Queen et al. refer to requires consideration in the context of the whole framework sequence from a single immunoglobulin (emphasis), whereas the homology the present invention refers to requires only consideration of each individual segments of FR1, FR2, FR3, and FR4. The idea of separating and treating the FR1, FR2 and

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FR3 as independent segments and entities is unprecedented, and it requires a functional understanding on the mechanism leading to immunogenicity of antibodies (or proteins) rather than simply a structural understanding. Details of which can be found in the previous argument, and in the specifications of this Application. See e.g. p. 14-16; p. 22-23 of the Specification.

Applicant (Leung et al. 1995. Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific, leukemia/lymphoma antibody, LL2. Mol. Immunol. 32:1413-1427) who is skilled in antibody engineering and independently came up with a similar idea as Ohtomo et al. at or around the same time as Ohtomo et al., has spent over 5 years to studying, investigating and experimenting in order to come up with the innovation of framework patching after a complete change of mindset on the issue of antibody immunogenicity. Accordingly, the transition of thinking from replacing the FR4 from a different sequence to the bold suggestion of replacing any of the FR1, FR2 and FR3 from different sequences cannot be obvious to one of ordinary skill in the art.

The Examiner further stated:

In addition, one of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced the claimed invention of replacing FR with human FR from any human antibody that has the most homology and reintroducing residues that influence binding in view of Ohtomo et al and Queen et al because Queen et al teach humanization by replacing FR with those that are the most homologous human FR and then reintroducing residues that influence antigen binding, are within 3 angstroms of a CDR or are rare at that position in the human FR.

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In response, Applicant respectfully traverses Examiner's above ground of rejection. Applicant maintains that the teachings of Queen et al. and Ohtomo et al. do not lead one of ordinary skill in the art to have motivation and reasonable expectation of success to produce the claimed invention. To summarize, the present invention includes but is not limited to the following embodiments: (1) using framework patching to identify human sequences with FR1, FR2, FR3 and FR4 having the desirable residues at critical positions (within 3 angstroms of a CDR or rare at that position, etc.) WITHOUT the need for back-mutation. This embodiment of the present invention bears no relevance on the teachings of Queen et al (emphasis). This embodiment should effectively eliminate the need for back-mutation. However, (2) when framework patching alone is insufficient in maintaining the binding affinity, back-mutation is allowed in another embodiment of the invention. By doing so, the number of back-mutated residues in the framework patched sequence will be less than that in the humanized sequence using one single immunoglobulin framework as taught by Queen et al.

The Examiner further stated:

In addition it would have been obvious to one of ordinary skill in the art to produce an antibody that had FR from different human antibodies because both Ohtomo et al and Queen teach that FR influence the CDRs and antigen binding and one wants the highest homology between the parent FR and the human FR that is to be substituted so one does not need to re-introduce many residues as taught by Ohtomo et al "the number of changes in the human FRs, however, should be minimized" (see page 414). In addition, it would have been obvious to produce the claimed invention because Ohtomo et al teach there approach is that human FRs are chosen based on the identification of the most homologous sequence (see page 414). Thus it would have been obvious to look at the FR and pick the most homologous regardless of

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whether they are from two or more antibody sequences.

In response, Applicant respectfully traverses Examiner's above ground of rejection. Applicant maintains that Ohtomo et al. focus on the identification of important residues that are needed to be reintroduced back to the human framework backbone in order to maintain affinity. The inclusion of a ND FR4 into EU FR1, FR2 and FR3 appeared to mimic a natural reshuffling phenomenon to obtain the highest sequence homology possible (note that Ohtomo et al. was careful to point out both EU and ND belong to the same member of the subgroup I to justify his choice). There was no intention, for example by Ohtomo et al. (as one skilled in the art is not able to think that to be possible and justifiable), to employ the mixing of FR1, FR2 and FR3 to avoid or minimize reintroduction of murine residues through such mixing. In the case of Ohtomo et al., their intention was to identify murine residues that are important for binding, and then choose a single human framework (for the whole light chain variable region), or sequence derived from a single human VH joined to a different JH (for the heavy chain variable region), and then reintroduce the murine residues that are identified to be important back to the chosen framework. No attempt to eliminate the need of back-mutation was ever made for such purpose. Page 414 of Ohtomo's publication stated "the number of changes in the human FRs, however, should be minimized", but does not provide a method different in principle from that of Queen et al., i.e. the identification of important murine residues to be back-mutated to the humanized design, to achieve such goal. Ohtomo et al offered an improved (but more labor-intensive) method to identify such residues to be back-mutated to supplement that of Queen et al. Ohtomo et al based on the approach of Queen et al had chosen REI as the light chain framework for CDR grafting, and identified five light chain FR residues at amino

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acids 27, 28, 29, 30, and 94 to be back mutated. It turned out that none of the back-mutation was sufficient in generating a function humanized antibody. In this anecdotal case, Ohtomo et al used hybrid VL and succeeded in locating Pro-46 within the FR2 to be the only residue important for binding, and requiring back-mutation. Other previously identified residues that were supposed to be important for binding and requiring back-mutation (based on Queen's approach) turned out to be unimportant, and no back-mutation at these sites was required. Queen et al. do this by the aid of computer modeling and a set of criteria to identify such residues, but the approach might potentially reintroduce more murine residues (as in the case of Ohtomo's experience in light chain humanization) than is necessary to maintain the affinity, thereby resulting in the problems of creating potentially new B and T epitopes. Ohtomo et al. adopt the method of Queen et al. and used computer modeling to assist in the identification of important residues for back-mutation, instead of finding stretches or segments of FRs to eliminate or reduce the need for back mutation.

For example, in the humanization of the heavy chain variable region, Ohtomo et al. chose EU FR1, FR2 and FR3 for CDR grafting, and had used computer modeling to identify amino acid residues at positions 27-30 (FN1K) of FR1 (Kabat's numbering) to be important for antigen binding; yet they chose to use the FR1 from EU which had four amino acid residues at positions 27-30 (GTFS) of the FR1 different from that of the murine sequence, instead of using, say, the FR1 from human 1B11'CL, 1H1'CL, 333'CL, 112'CL, 126'CL, 115'CL, etc. (Kabat's data base), all of which has the sequence at positions 27-30 of FNFS. This would be the obvious choice as these FR1 sequences contain identical amino acid residues at positions 27 and 28 (FN), and only two mutations (FS conversion to 1K) are required, instead of four (GTFS conversion into FN1K), as

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in the case of Ohtomo et al. Ohtomo's idea of minimizing the number of changes in the human FRs should be done taking FR1, FR2 and FR3 as one inseparable structure. Ohtomo et al. also emphasized that both EU and ND sequences are members of subgroup I (page 412) to justify the joining of the FR1, FR2 and FR3 of EU to the FR4 of ND as natural and canonical. It clearly demonstrated that Ohtomo et al. did not consider the assortment of FR segments to be a possible method of minimizing the number of changes in the human FRs.

Applicant maintains that there is no motivation to combine what is disclosed in Queen et al. with the background of Ohtomo et al. Even if Ohtomo et al. reported the use of the FR1, FR2 and FR3 from one antibody, and the FR4 from a different antibody in the construction of a heavy chain variable region, the homology Ohtomo et al. and Queen et al. refer to requires consideration in the context of the whole framework sequence from a single immunoglobulin (emphasis), whereas the homology the present invention refers to requires only consideration of each individual segments of FR1, FR2, FR3, and FR4. The idea of separating and treating the FR1, FR2 and FR3 as independent segments and entities is unprecedented, and it requires a functional understanding on the mechanism leading to immunogenicity of antibodies (or proteins) rather than simply a structural understanding.

Accordingly, Ohtomo et al. further in view of Queen et al. does not render the claimed invention obvious. Therefore, Applicants respectfully request the reconsideration and withdrawal of the above grounds of rejection.

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CONCLUSION

Applicant respectfully contends that the Examiner's objections and/or rejections raised in this Office action have been fully addressed, and therefore this Application is in full compliance with all requirements. Accordingly, Applicant respectfully urges the Examiner to reconsider and withdraw all objections and/or rejections in this Office action and place this application in conditions for allowance.

If a telephone interview would be of assistance in advancing prosecution of the subject application, Applicant's undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is given to charge the amount of any such fee to Deposit Account No. 50-1891.

Respectfully submitted,

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